

*Courtesy Copy of Pending Claims for
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1. (Amended) A method of identifying a recombinant herbicide tolerance nucleic acid which can confer tolerance to an herbicide upon a cell in which the recombinant herbicide tolerance nucleic acid is present, the method comprising:

(i) providing a plurality of nucleic acid segments, which nucleic acid segments are derived from a plurality of variant forms of one or more parental nucleic acids, wherein one or more of the parental nucleic acids encode a polypeptide with an herbicide tolerance activity;

(ii) recombining the plurality of nucleic acid segments to produce a library of recombinant nucleic acids; and,

(iii) screening the library to identify at least one recombinant herbicide tolerance nucleic acid which encodes a polypeptide with an activity that confers herbicide tolerance to a cell.

2. (Amended) The method of claim 1, wherein the at least one recombinant herbicide tolerance nucleic acid encodes a polypeptide with a distinct or improved herbicide tolerance activity compared to a parental nucleic acid.

3. (Amended) The method of claim 1, wherein a plurality of parental nucleic acids encode polypeptides with herbicide tolerance activities, which herbicide tolerance activities are the same or different between polypeptides.

4. (Amended) A method of identifying a recombinant herbicide tolerance nucleic acid which can confer tolerance to an herbicide upon a cell in which the recombinant herbicide tolerance nucleic acid is present, the method comprising:

(i) providing a plurality of nucleic acid segments, which nucleic acid segments are derived from a plurality of variant forms of one or more parental nucleic acids, wherein the one or more parental nucleic acids encode a polypeptide lacking herbicide tolerance activity;

(ii) recombining the plurality of nucleic acid segments to produce a library of recombinant nucleic acids; and,

(iii) screening the library to identify at least one recombinant herbicide tolerance nucleic acid which encodes a polypeptide with an activity that confers herbicide tolerance to a cell.

5. (Amended) The method of claim 1 or 4, wherein one or more parental nucleic acids encode polypeptides which are functionally or structurally similar to an herbicide target protein.

6. (Amended) The method of claim 1 or 4, wherein the plurality of variant forms comprises allelic or interspecific variants of one or more parental nucleic acids.

7. (Amended) The method of claim 1 or 4, wherein the plurality of variant forms is produced by synthesizing a plurality of nucleic acids homologous to the one or more parental nucleic acids.

8. (Amended) The method of claim 1 or 4, wherein the plurality of variant forms is produced by error-prone transcription of the one or more parental nucleic acids or by replication of the one or more parental nucleic acids in a mutator cell strain.

9. (Amended) The method of claim 1 or 4, wherein the one or more parental nucleic acids encode a polypeptide or polypeptide fragment selected from the group consisting of: a P450 monooxygenase polypeptide, a glutathione sulfur transferase polypeptide, a homoglutathione sulfur transferase polypeptide, a glyphosate oxidase polypeptide, a phosphinothricin acetyl transferase polypeptide, a dichlorophenoxyacetate monooxygenase polypeptide, an acetolactate synthase polypeptide, a protoporphyrinogen oxidase polypeptide, a 5-enolpyruvylshikimate-3-phosphate synthase polypeptide, and a UDP-N-acetylglucosamine enolpyruvyltransferase polypeptide.

10. (As filed) The method of claim 9, wherein the parental nucleic acid is selected from the group consisting of: a P450 monooxygenase gene from corn or wheat, a glutathione sulfur transferase gene from corn, a homoglutathione sulfur transferase gene from soybean, a glyphosate oxidase gene from a bacteria, a phosphinothricin acetyl transferase gene from a bacteria, a dichlorophenoxyacetate monooxygenase gene from a bacteria, an acetolactate synthase gene from a plant, a protoporphyrinogen oxidase gene

from a plant, a protoporphyrinogen oxidase gene from an alga, an enolpyruvylshikimate-3-phosphate synthase gene from a bacteria, a enolpyruvylshikimate-3-phosphate synthase gene from a plant, and a UDP-N-acetylglucosamine enolpyruvyltransferase gene from a bacteria.

11. (As filed) The method of claim 5, wherein the parental nucleic acid encodes a UDP-N-acetylglucosamine enolpyruvyltransferase, and wherein the herbicide is glyphosate.

12. (Amended) The method of claim 1 or 4, wherein the library comprises at least one recombinant nucleic acid produced by recombining a plurality of variant forms of one or more parental nucleic acids selected from the group consisting of:

a P450 monooxygenase nucleic acid, a homoglutathione sulfur transferase nucleic acid, a glutathione sulfur transferase nucleic acid, a glyphosate oxidase nucleic acid, a phosphinothrin acetyl transferase nucleic acid, a dichlorophenoxyacetate monooxygenase nucleic acid, an acetolactate synthase nucleic acid, a enolpyruvylshikimate-3-phosphate synthase nucleic acid, and a UDP-N-acetylglucosamine enolpyruvyltransferase nucleic acid.

13. (Amended) The method of claim 1 or 4, wherein the screening comprises a step selected from the group consisting of:

- (a) screening for oxidation of an herbicide;
- (b) screening for glutathione conjugation to an herbicide or to a metabolite of an herbicide; and,
- (c) screening for a homoglutathione conjugation to an herbicide or to a metabolite of an herbicide.

14. (Amended) The method of claim 1 or 4, wherein the library of recombinant nucleic acids is present in a population of cells.

15. (As filed) The method of claim 14, wherein the screening comprises growing the population of cells in or on a medium comprising the herbicide and detecting

a physical difference between the herbicide and a modified form of the herbicide produced by the cells.

16. (As filed) The method of claim 15, wherein the physical difference between the herbicide and the modified form of the herbicide is detected by a difference in fluorescence or absorbance between the herbicide and the modified form of the herbicide.

17. (As filed) The method of claim 16, wherein the herbicide is dicamba, the recombinant herbicide tolerance nucleic acid encodes a dicamba oxidation activity, and the cells are screened for dicamba oxidation by fluorescence of an oxidized form of dicamba.

18. (As filed) The method of claim 14, wherein the screening comprises growing the population of cells in or on a medium comprising the herbicide and selecting for enhanced growth of the cells in the presence of the herbicide.

19. (As filed) The method of claim 18, wherein enhanced growth of the cells requires the activity encoded by the recombinant herbicide tolerance nucleic acid.

20. (As filed) The method of claim 19, wherein enhanced growth of the cells requires the product of the reaction of the herbicide by the activity encoded by the recombinant herbicide tolerance nucleic acid.

21. (As filed) The method of claim 20, wherein the cell is an Mpu^+ strain of bacteria, the herbicide is glyphosate, and the recombinant herbicide tolerance nucleic acid encodes an activity that catalyses the conversion of glyphosate to aminomethylphosphonate.

22. (As filed) The method of claim 19, wherein the cells are an $AroA^-$ strain of bacteria, the herbicide is glyphosate, and the recombinant herbicide tolerance nucleic acid encodes an activity which catalyses the conversion of phosphoenolpyruvate plus shikimate 3-phosphate to 5-enolpyruvylshikimate-3-phosphate.

23. (Amended) The method of claim 1 or 4, the method further comprising screening the library for one or more additional activity that confers tolerance to one or more additional herbicides.

24. (Amended) The method of claim 1 or 4, wherein the recombining is performed in a population of cells.

25. (Amended) The method of claim 24, further comprising:

(a) recombining at least one nucleic acid encoding a polypeptide with an herbicide tolerance activity by introducing a second plurality of nucleic acid segments into the population of cells, such that at least one introduced nucleic acid segment recombines with a nucleic acid present in a member of the population of cells to produce modified cells or recombining at least one nucleic acid that encodes a polypeptide with an herbicide tolerance activity between at least two members of the population of cells to produce modified cells.

26. (Amended) The method of claim 25, further comprising:

(b) recombining and screening the modified cells to produce further modified cells that have evolved an additional distinct or improved herbicide tolerance activity.

27. (Amended) The method of claim 26, further comprising:

repeating (a) or (b) until the further modified cells have acquired an additional distinct or improved herbicide tolerance activity.

28. (Amended) The method of claim 1 or 4, wherein the method further comprises:

(iv) recombining at least one recombinant herbicide tolerance nucleic acid with an additional nucleic acid, wherein the additional nucleic acid is the same or different from one or more of the variant forms of (i), to produce an additional library of recombinant nucleic acids; and

(v) screening the additional library to identify at least one additional recombinant herbicide tolerance nucleic acid that encodes a polypeptide with an

additional improved herbicide tolerance activity compared to a non-recombinant herbicide tolerance nucleic acid; and, optionally repeating (iv) and (v).

29. (Amended) The method of claim 28, wherein the additional recombinant herbicide tolerance nucleic acid encodes a polypeptide with two or more distinct or improved herbicide tolerance activities.

30. (Amended) The method of claim 1 or 4, wherein the library of recombinant nucleic acids is present in bacterial cells and the screening of step (iii) comprises:

pooling a plurality of cells each comprising a separate member of the library produced in step (ii);

screening the resulting pooled cells for a recombinant herbicide tolerance nucleic acid that encodes a polypeptide with a distinct or improved herbicide tolerance activity compared to a non-recombinant herbicide tolerance nucleic acid; and,

isolating the nucleic acid encoding the polypeptide with the distinct or improved herbicide tolerance activity.

31. (Amended) The method of claim 1 or 4, comprising screening for a distinct or improved herbicide tolerance activity, which activity is selected from the group consisting of: an increase in ability to metabolize an herbicide; an increase in the range of herbicides to which the activity confers tolerance; an increase in expression level compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in susceptibility to inhibition by an herbicide compared to that of an activity encoded by the parental nucleic acid; a decrease in susceptibility to protease cleavage compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in susceptibility to high or low pH levels compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in susceptibility to high or low temperatures compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in toxicity to a host plant compared to that of a polypeptide encoded by the selected nucleic acid; and any combination of two or more activities selected therefrom.

32. (Amended) The method of claim 1 or 4, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant.

33. (Amended) The method of claim 1 or 4, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant and testing the resulting transduced plant for tolerance to an herbicide.

34. (Amended) The method of claim 1 or 4, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant and breeding the plant with another plant strain of the same species, and selecting resultant offspring for tolerance to an herbicide.

35. (Amended) A library of recombinant nucleic acids made by the method of claim 1 or 4.

36. (As filed) The library of claim 35, wherein the library is a phage display library.

37. (Amended) A recombinant herbicide tolerance nucleic acid made by the method of claim 1 or 4.

38. (New) The method of claim 1 or 4, further comprising isolating or recovering the at least one identified recombinant herbicide tolerance nucleic acid which encodes a polypeptide with an activity that confers herbicide tolerance.